DISCOVERY

57(303), March, 2021

To Cite:
Shiv Nandan Sah, Jeena Katwal, Arjun Ghimire, Ranjit Kumar Sah, Pradeep Kumar Sah. Detection of Antibiotic Residues in Broiler Chicken Meat Sold in Dharan Sub-metropolitan City, Nepal. *Discovery*, 2021, 57(303), 305-311

Author Affiliation:
[1]Department of Microbiology, Central Campus of Technology, Tribhuvan University, Dharan, Sunsari, Nepal
[2]Department of Microbiology, Ram Swaroop Ram Sagar Multiple Campus, Tribhuvan University, Janakpurdham, Dhanusha, Nepal
[3]Department of Microbiology, Tri-Chandra Multiple Campus, Ghantaghar, Kathmandu, Tribhuvan University, Nepal

✉**Corresponding author:**
Shiv Nandan Sah, Department of Microbiology, Central Campus of Technology, Hattisar, Dharan, Tribhuvan University, Sunsari, Nepal.
Email: sahshivnandan96@gmail.com

Peer-Review History
Received: 17 January 2021
Reviewed & Revised: 19/January/2021 to 16/February/2021
Accepted: 17 February 2021
Published: March 2021

Peer-Review Model
External peer-review was done through double-blind method.

Detection of Antibiotic Residues in Broiler Chicken Meat Sold in Dharan Sub-metropolitan City, Nepal

Shiv Nandan Sah[1]✉, Jeena Katwal[1], Arjun Ghimire[1], Ranjit Kumar Sah[2], Pradeep Kumar Sah[3]

ABSTRACT

Background: The antibiotic residues in poultry meat can cause public health hazards such as sensitivity to antibiotics, allergic reactions, a mutation in cells, imbalance of intestinal microbiota, and bacterial resistance to antibiotics. *Objectives:* This study aimed to detect antibiotic residue in Broiler chicken meat collected from the retail shop of Dharan, Province-1 (Nepal) from April to June 2019. *Method:* A total of 60 chicken meat samples (20 muscle samples, 20 liver samples, and 20 gizzard samples) were collected in plastic bags and tested for detection of antibiotic residues against *E. coli* and *S. aureus* by using Agar well diffusion method on MHA through antibiotic susceptibility test and TLC in the microbiology laboratory in Central Campus of Technology, Dharan, Tribhuvan University, Nepal. *Result:* Among 20 muscle samples tested, 3 (15 %) were found to contain antibiotics; While among 20 liver samples tested, 6 (25 %) were found to be positive. But, out of 20 gizzard samples tested, 1 (5 %) were found to be positive. In the microbial susceptibility test, both test bacteria namely *E. coli* and *S. aureus* were inhibited in 7 (11.67%) and 3 (5%) samples respectively among the total of 10 positive samples. In this study, among 7 samples in which *E. coli* was inhibited, only 3 were taken for the TLC method. In TLC, R_f values of all three samples loaded were calculated as 0.496, 0.464, and 0.456 respectively. *Conclusion:* This study revealed the presence of antibiotic residue in broiler chicken meat sold in Dharan which demands strict rules and regulations as well as surveillance from the concerned authorities.

Keywords: Antibiotic residue, Broiler chicken meat, well diffusion method, MHA, TLC

INTRODUCTION

Meat, one of the most important constituents of the human diet, provides protein, energy, vitamins, and minerals. However, meat could also become a source of health hazards if it contains excess fats or harmful materials such as toxins or residues of chemical agents.[1] A broad range of antibiotics is used in poultry not only to treat diseases but also to maintain health, promote growth, and enhance feed efficiency. [2, 3,34] Existence of the antibiotic residues in foodstuff can make

DISCOVERY SCIENTIFIC SOCIETY

hazards to human health such as sensitivity to antibiotics, allergic reactions, and imbalance of intestinal microbiota.[4]

Antibiotic residues such as Oxytetracycline and Eurofloxacin have been detected above the maximum residue level (MRL) in chicken tissues[5]. Antibiotic growth promoters are found to suppress the gut bacteria which leave more nutrients for chickens to be absorbed.[6] The antibiotics that have been given to poultry and livestock production were deposited in the liver, kidney, muscle, and bones surpassing the Maximum Residue Limit.[7] The use of antibiotics in food-producing animals increases the risk of antibiotic resistance and allergic reactions to antibiotics[8] which may eventually become a part of the human diet.[9]

The emerged resistant bacteria may not be harmful to the animal, but can be pathogenic to humans and may complicate treatment.[10] Hence, the protection of public health against possible harmful effects of antibiotic residues is a burning issue in the whole world.[11] This study was carried out for the detection of antibiotic residues in chicken meat sold in Dharan.

MATERIALS AND METHODS

Sample Collection

A total of 60 chicken meat samples (20 muscles, 20 livers, and 20 gizzards) were collected from respective 20 chicken meat of local market of Dharan (Latitude: 26° 48' 44.93" N; Longitude: 87° 17' 0.78" E) Sunsari (Nepal) in April to June 2019. The study site is a town of Province number 1 of Nepal locating between the hills and Terai region at the altitude of 349 m. From each chicken sample, 3 tissue samples (1 muscle tissue, 1 liver tissue, and 1 gizzard tissue) were taken and transported to the microbiology laboratory of the department of microbiology, Central Campus of Technology, Dharan, Tribhuvan University, Nepal. In the case of delay of processing, the samples were preserved in the deep freeze at the temperature -4°C.

Extraction of antibiotic from a meat sample

10 grams of each sample was first crushed and ground in a sterile mortar with the help of a sterile pestle and the 10 ml of phosphate buffer was added. Then it was mixed by vortexing. The solution was transferred to centrifuge tubes and centrifuged at 700 rpm for 10 minutes. The clear supernatant was transferred to fresh glass test tubes and was incubated in an incubator at 37 °C for 30 minutes (Kirubaharan et al 1998).[12]

Evaluation of the antibacterial activity of chicken meat extract

The test bacterial inoculums (0.5 Mcfarland standards) of *S. aureus* and *E. coli* were evenly spread on sterile MHA media plates separately with the help of sterile cotton swab. Then the medium was allowed to dry for 3-5 minutes to allow for absorption of excess moisture. Three wells were made with the help of a sterile cork borer and filled with extracts (clear supernatant) of gizzard, liver, and muscle respectively by using a micropipette. The plates were then incubated in an incubator at 37° C for 24 to 48 hrs (Javadi et al 2011).[4]

Thin Layer Chromatography

This technique was used to separate non-volatile mixtures. It was performed on a sheet of glass coated with a thin layer of adsorbent material (i.e. silica gel) as the stationary phase.

The same supernatant sample extracted for the microbiological test was used for this process. The supernatant was filtered with the help of Whatman Filter Paper and funnel. The filtrate was collected in another clean tube and an equal amount of di-ethyl ether was added and left at the room temperature for 10 minutes. The final extracts were carefully poured and collected in a tube.

RESULTS

Sample collection and transportation

During three months, 60 chicken meat samples (20 muscles, 20 livers, and 20 gizzards) were collected from different shops for the detection of antibiotic residues in those samples. Samples were processed immediately as soon as possible after reaching to the Microbiology laboratory of Central Campus of Technology, Tribhuvan University, Nepal, otherwise preserved at -4° C.

Bacterial Growth Inhibition Assay

In this study, the test bacteria used were *Staphylococcus aureus* and *Escherichia coli*. The test bacteria were spread in separate plates containing the cultural medium and the results were noted for each of the plates. Both test bacteria namely *E. coli* (figure 1) and *S. aureus* (figure 2) were inhibited in 7 (11.67%) and 3 (5%) samples among a total of 60 samples used (Table 1).

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

Table 1: Susceptibility of test micro-organisms against raw chicken meat samples

Test organisms	Total samples	Samples showing Inhibition zones	% of samples showing inhibition zones
Escherichia coli	60	7	11.67
Staphylococcus aureus	60	3	5

Among 20 muscle samples tested, 3 (15 %) showed antibacterial effects While out of 20 liver samples tested, 6 (25 %) shoed antibacterial effects. But, among 20 gizzard samples tested, only 1 (5 %) antibacterial effect.

Table 2: Susceptibility of test micro-organisms against broiler chicken muscle, liver, and gizzard samples

Target organ	Total no. of samples	Samples showing antibacterial activity	% of samples showing antibacterial activity
Muscle	20	3	15
Liver	20	6	25
Gizzard	20	1	5

Figure 1: Microbial susceptibility test showing inhibition zone in *E. coli*

Figure 2: Microbial susceptibility test showing inhibition zone in *S. aureus*

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

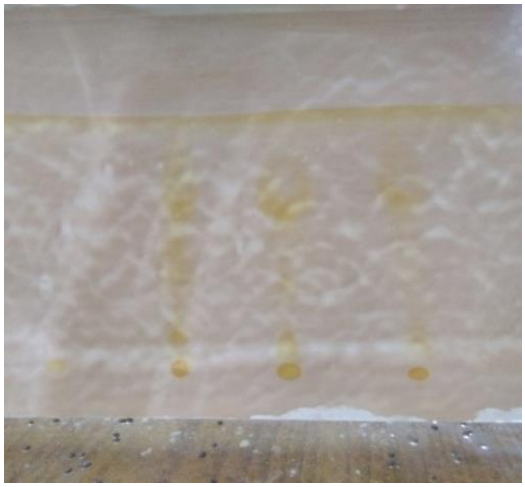

Figure 3: TLC of antibiotic residues extracted from chicken meat samples

Thin Layer Chromatography

In this study, out of 10 positive samples, only 3 were taken for the TLC method (figure 3). All three samples inhibited *E. coli* as seen in the microbiological susceptible test. By using the formula, R_f values all three samples (L_6, L_{12}, and L_{15}) loaded were calculated as 0.496, 0.464, and 0.456 respectively (Table 3).

Table 3: Calculation of Rf value of extracted antibiotic from chicken meat in TLC

Samples code	Distance traveled by the solvent	Distance traveled by sample spots	R_f value
L_6	12.5	6.2	0.496
L_{12}	12.5	5.8	0.464
L_{15}	12.5	5.7	0.456

DISCUSSION

Nowadays, many antibiotics are widely used by humans[13] because of their effectiveness and easy access for preventing and treating various diseases as well as for uplifting growth (growth stimulant) in animals specially used as food.[11,14] Antibiotics are given periodically during the lifetime of the broilers to promote growth and for production by preventing bacterial disease in livestock.[15] Since broiler chickens are the largest sources of meat across the world, market demand has put the farmers under continuous pressure to produce chickens in the shortest period time with the maximum output.

Red meat is not very friendly to human consumption. Moreover, it also raises the levels of cholesterols, fat, uric acid, hormonal secretion.[16] It also allows the body to become an open ground for a variety of cancer like cancer of the colon, stomach, intestine, pancreas even breast, especially due to high levels of uric acid that increase with the consumption of red meat.[17]

In contrast, the white meat, generally found within the breast muscles of the birds, is used for quick bursts of power, which require little of the meat darkening myoglobin. White meat is far safer in this respect. It has been found by medical practitioners that white meat especially the lean variety does not have too high levels of fats and so does not contribute to cause cardiovascular diseases.[18]

Common diseases such as typhoid, Mycotoxicosis, *E. coli* infections, Coccidiosis, Salmonellosis, Enteritis, Ascites, Newcastle disease, etc., affect poultry growth and production and have become a factor for the economic losses due to high mortality among the flocks.[9]

In this study, a total of 60 chicken meat samples were analyzed. Among 60 chicken meat (20 muscles, 20 livers, and 20 gizzards) samples, 10 (16.67%) were found to be positive for antibiotic residues. Two test bacteria namely *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) were used as test bacteria. Among the two bacterial cultures used in this study, *Escherichia coli* were inhibited in 7 samples (11.67 %) followed by *Staphylococcus aureus* in 3 samples (5 %). In this study, 3 (15 %) among 20 muscle samples, 6 (25%) among 20 liver samples, and 1(5%) among 20 gizzard samples were found to contain antibiotics.

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

According to this study, among the three different tissue sample liver showed the highest content of antibiotic residue followed by muscles and gizzards.

A Similar study in Kathmandu valley clearly showed the presence of antibiotic residue in the sampled poultry tissue. It was found that 13% of 60 samples were found to be positive and 87% were found to be negative. Out of 8 positive tissue samples, 5 muscle samples, and 3 liver samples were found positive.[6]

A similar study was done in the Kavre and Kailali districts of Nepal by using the Kit Test method, 22% of the samples were found positive.[19] The finding of chicken meat samples containing antibiotic residue in our study is lower than some of the similar studies; 40%[20] and 27%.[21] The differences in results could be due to various reasons. The use of different bacteria as test organism and their susceptibility to different antibiotics, due to the inconsistencies in sample size, the variation of the practice of antibiotic use in different locations, differences in the methods for detection. [6]

Screening of antibiotic residue in food products from animal origin began mainly with the introduction of antibacterial therapy in veterinary practice. There are several screening methods available which are simple and reliable for the detection of antibiotics. This is an essential tool in assuring the safety of food products.[22]

Microbial inhibitions assays were the earliest methods used for the detection of antibiotic residues and are still in use because they are inexpensive and can cover the entire antibiotic spectrum but are less specific than other tests.[23] The foodstuffs which contain a higher concentration of antibiotics that is beyond the tolerance levels must be verified by highly selective and sufficiently sensitive chemical methods.[24]

In Nepal, the majority of drugs are used without any hindrances whatsoever in a lump sum amount, and upkeep of withdrawal period and evaluation of residue in meat and kinds of milk are not effectively tracked by the government and private sectors.[25]

Since the main consumers of animal products are humans, several serious health problems have been detected in them due to the intake of those animal products which are continuously exposed to antibiotics. Humans may have an allergic reaction, spreading of drug-resistant micro-organisms, carcinogenic effect, and potentially harmful effects on intestinal micro-flora.[26] Some antibiotics are directly toxic, like chloramphenicol which causes aplastic anemia, while allergic reactions and toxic side effects may have fatal consequences.[27]

Due to malnutrition, medication or frequent occurrence of disease may result in immuno-suppression in poultry animals leading to increased susceptibility to potential pathogens. To control or manage such frequent occurring diseases in the poultry animals, poultry farmers prefer the use of antibiotics.[11]

Since the population of the world is increasing rapidly, to ensure enough food production to feed the enlarging world population, a high amount of antibiotics have been supplied to the poultry animals by the poultry farmers as they are pushed towards a more intensive production. Antibiotics used for intensive production of poultry animals lead to an increase of bacterial infections and consequently a higher therapeutic and even prophylactic use of antibiotics. The use of antibiotics in an excessive way results in the presence of residues of these substances or their metabolites in food from animal origin.[28] The most commonly sold antimicrobial classes in the major livestock, especially in poultry production in 15 countries from Europe, Asia, and Australia, were penicillins, tetracyclines, macrolides, and aminoglycosides, especially since each of these classes has been in use for more than 50 years.[29]

Antimicrobial residues in food of animal sources have gained big attention in developed countries for ensuring food safety. Many developed countries have been reported conducting monitoring programs to avoid antimicrobial residue in the food of animal origin. [30] The protection of public health against the possible harmful effects of antibiotic residues has become the major concern to deal with. Therefore, a large number of samples are needed for the analysis of the indivisible use of antibiotics. [11]

Poultry farmers mostly slaughter their animals during treatment with antibiotics or before the withdrawal period so that a high percentage of antibiotic residues are found in chicken meat. A similar study was carried out by Trivedi (2012).[11] The non-compliance to the withdrawal period by farmers could be associated with many reasons including fear of economic losses. The implementation of the withdrawal period may cause a delay in the sale of chicken leading to huge losses to the poultry farmers. The other reason could be a lack of awareness to farmers on the possible side effects of antimicrobials and other drugs to animals and humans.

Thin-layer chromatography is a sensitive and highly reliable method for the qualitative and quantitative detection of antibiotic residues in meat. As TLC is less time consuming, low cost and can be performed with a less complicated technique it has a wide application in various analyses. It also has a wide application in identifying impurities in a compound .[11]

Dependence on medically important antibiotics used in animals should be reduced. Alternatives such as in-feed enzymes, competitive exclusion products, probiotics, and infection control measures should be used instead of medically important antibiotics .[31]

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

There is an immediate warning on the potential effects on public health unless government regulates and implements the use of antimicrobials.[32] The presence of residue from veterinary medicinal products in foods of animal origin could jeopardize international trade.[33]

CONCLUSION

This study revealed the presence of antibiotic residue in broiler chicken meat sold in Dharan which demands strict rules and regulations as well as surveillance from the concerned authorities. Dependence on medically important antibiotics used in animals should be reduced. The alternatives such as in-feed enzymes, competitive exclusion products, probiotics and infection control measures should be used instead of medically important antibiotics. Microbial growth inhibition assay results showed that out of two test micro-organisms, the highest amount of raw samples of chicken meat inhibited *E. coli* followed by *S. aureus.*

Limitation

This study was carried out in Dharan for only three months (April to June 2019). This study did not identify the antibiotic present in various chicken samples with their molecular structure.

Acknowledgement

The authors would like to thank the Department of Microbiology, Central Campus of Technology, Dharan, Tribhuvan University, Nepal, for providing laboratory facilities to complete this research work.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding:

There are no funding sources for this paper.

Ethical approval

This article does not contain any studies with human participants performed by any of the authors.

Data and materials availability:

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Mahgoub O, Kadim IT, Mothershaw A, Alzadjali SA, Annamalai K. Use of enzyme linked immunosorbent assay for detection of antibiotic and anabolic residues in goat and sheep meat. World journal of agricultural sciences, 2006; 2(3): 298-302.
2. Okerman L, Noppe H, Cornet V, Zutter LD. Microbiological detection of 10 quinolone antibiotic residues and its application to artificially contaminated poultry samples. Food Additives & Contaminants, 2007; 24(3):252-257.
3. Nisha AR. Antibiotic Residues - A global health hazard. Veterinary World, 2008; 1(12):375-377.
4. Javadi A, Mirzaie H, Khatibi SA. Effect of roasting, boiling and microwaving cooking methods on Enrofloxacin residues in edible products. Journal of Chromatography B: Biomedical Sciences and Applications, 2011; 667(1):1-40.
5. Karmi M. Detection and presumptive identification of antibiotic residues in poultry meat by using FPT. Global J Pharmacology, 2014; 8(2):160–165.
6. Sapkota R, Raut R, Khanal S, Gyawali M, Sahi D. Screening of antibiotic residue in poultry in Kathmandu valley of Nepal: A cross-sectional study. Arch Pharma Sci., 2019; 3:79-81. DOI: dx.doi.org/10.29328/journal.apps.1001017
7. Sarker YA, Hasan MM, Paul TK, Rashid SZ, Alam MN, Sikder MH. Screening of antibiotic residues in chicken meat in Bangladesh by thin layer chromatography. Journal of Advance Veterinary and Animal Research, 2018; 5(2):140-145.
8. Gärd A, Johansson J. Project report: Development of sample workup and analysis scheme for determination of chloramphenicol in shrimp feed using HPLC. Vmea University, Sweden, 2004.
9. Mund MD, Khan UH, Tahir U, Mustafa BE, Fayyaz A. Antimicrobial drug residues in poultry products and implications on public health: A review. Int J Food Properties, 2017; 20(7):1433-1446.

10. Myaing TT. Public Health Aspects of Antibiotic Residues in Food of Animal Origin. *Myanmar Livestock Breeding Journal.2003*:32-36.
11. Trivedi SM. Detection of Antibiotics Residues in muscle, liver and kidney of Chicken (Ph D Thesis) Jawalapur. M.P.P.C.V.V., 2012
12. Kirubaharan JJ, Palaniswami KS, Anubukumar K, Mohanasubramaniam B. In-vitro studies on antimicrobial effect of crude garlic extract on *Escherichia coli*. Indian Veterinary Journal, 1999; 76(9): 797-799.
13. Riaz M, Khurram M, Akhtar S, Park S, Ismail A. Antibiotic Residues in chicken meat: Global Prevalance Threats, and Decontamination Strategies: A Review; Journal of food protection, 2018;81(4):619-627
14. Dixon SN. Veterinary Drug Residues. In: Watson DH, editor. Food Chemical Safety; Volume 1: Contaminants. Cambridge, UK: Woodhead Publishing Ltd.; 2001.p.109-147
15. Gustafson RH, Bowen RE (1997). Antibiotic use in Animal Agriculture. Journal of applied microbiology, 1997; 83(5): 531-541.
16. Alison JM, Emeir MM, Geraldine JC, Bruce WM, Julie MWW, Maxine PB, Anna MF. Red meat consumption: An overview of the risks and benefits. Meat Science, 2010; 84(1):1-13.
17. Sajid A, Kashif N, Kifayat N, Ahmad S. Detection of antibiotic residues in poultry meat. Pak. J. Pharm. Sci., 2016; 29 (5):1691-1694.
18. Frank BH, Walter C, Willett. Optimal diets for prevention of coronary heart disease. The Journal of the American Medical Association, 2002; 288(20): 2569-2578.
19. Raut R, Mandal RK, Kaphle K, Pant D, Nepali S, Shreshtha A. Assessment of Antibiotic Residues in the Marketed Meat of Kailali and Kavre of Nepal. International J Applied Sci Biotechnol., 2017; 5(3): 386-389.
20. Ezenduka EV, Ike OS, Anaelom NJ. Rapid detection of antimicrobial residues in poultry: A consequence of non-prudent use of antimicrobials. Health, 2014; 6(2):149-152.
21. Elnasri A, Salman M, Rade SA, El Mohammed Kolo B, Geidam YA et al. Screening of Antibiotic Residues in Poultry Liver, Kidney and Muscle in Khartoum State, Sudan Sample collection. Bas J Vet Res, 2014; 14:43–51.
22. Mitchell JM, Griffiths MW, McEwen SA, McNab WB, Yee AJ. Antimicrobial Drug Residues in Milk and Meat: Causes, Concerns, Prevalence, Regulations, Tests and Test performance. Journal of food protection, 1998; 61(6): 742-756.
23. Hind AE, Adil MS, Samah AR. Screening of Antibiotic Residues in Poultry Liver, Kidney and Muscle in Khartoum State, Sudan. Journal of Applied and Industrial Sciences. 2014; 2(3):116–122.
24. Leal C, Codony R, Compano R, Granados M, Prats M. Determination of macrolides antibiotics by liquid chromatography. Journal of Chromatography A, 2001; 910(2):285-290.
25. Thapaliya M, Karki TB, Sedai D. Sulfonamides and Penicillin Residue in Market Milk. Journal of Food Science and Technology, Nepal, 2013; 8: 60–64
26. Pavlov A, Lashev L, Rusev V. Studies on the Residue Levels of Tetramycine in stored Poultry Products. Trakia Journal of Science, 2006; 3: 20-22.
27. Popelka P, Nagy J, Popelka P, Marcincak S, Jevinova P, Hussein K. Comparison of BSDA and PREMI test sensitivity to penicillin standards in poultry meat and after administration of Amuril plv. sol. Folia Veterinaria, 2003; 47:139–141.
28. Muaz K, Riaz M, Akhtar S, Park S and Ismail A. Antibiotic Residues in Chicken Meat: Global Prevalence, Threats, and Decontamination Strategies: A Review. *Journal Food Prote*ction, 2018; 81 (4): 619–627. doi: https://doi.org/10.4315/0362-028X.JFP-17-086
29. Page SW, Gautier P. Use of antimicrobial agents in livestock. Revue Scientifique et Technique-(International office of Epizootics), 2012; 31(1):145-188.
30. Ellis RL. Development of veterinary drug residue controls by the Codex Alimentarius Commission: a review. Food Additives and Contaminants: Part A, 2008; 25 (12), 1432-1438, DOI: 10.1080/02652030802267405
31. Moe TS, Hla TT, Mon HM. Detection of Antibiotic Residues in Broiler Chicken Meat. Journal of Medical Science and Clinical Research, 2018; 6 (7):326-332.
32. Wise R. Antimicrobial resistance: Priorities for action. Journal of Antimicrob Chemotherapy, 2002; 49(4): 585-586.
33. Tadesse T. Public Health Impacts of Antibiotic Residues in Foods of Animal Origin: A Review. Public Policy and Administration Research, 2017; 7(10): 6-11.
34. Saranya S, Kalaikannan A, Santhi D, Abinaya JB. Chemical Residues – A concern for Meat consumers. *International Journal of Adulteration*, 2020; 4: e1ijad3012

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS